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BCL-2 Oncoprotein (p26) in Splenic Lymphoma With Villous Lymphocytes: A Comparative Study With Other Chronic B-Cell Disorders

Margarida Lima,* Maria dos Anjos Teixeira, Maria Luís Queirós, Ana Helena Ribeiro dos Santos, and Benvindo Justiça

Service of Clinical Hematology, S. Antonio's Hospital, Porto, Portugal

We compared the expression of the BCL-2 oncoprotein (p26) on B cells from 24 patients with splenic lymphoma with circulating villous lymphocytes (SLVL) with that observed on normal, mature B lymphocytes and on neoplastic B cells from 91 patients with other chronic B-cell malignancies. SLVL B cells showed levels of p26 intermediate between those found on normal B lymphocytes and on neoplastic B cells from patients with other chronic B-cell lymphoproliferative disorders. Am. J. Hematol. 56:122–125, 1997.

Key words: BCL-2; p26; apoptosis; SLVL

INTRODUCTION

The BCL-2 oncogene has been found to suppress apoptosis by encoding for a 26-Kd protein (p26), and its role has been already established in various physiological and pathological states [1]. Upregulation of BCL-2 has been implicated in the genesis of various B-cell neoplasms [2–4] and may influence the response to chemotherapy and prognosis [5].

Splenic lymphoma with circulating villous lymphocytes (SLVL) is a chronic B-cell lymphoproliferative disorder whose clinical and laboratorial features, natural history, and response to therapy were previously characterized in detail [6–8]. Patients with SLVL usually present with splenomegaly and moderate lymphocytosis, with little or no lymphadenopathy. Moderate anemia and thrombocytopenia are frequent findings. A discrete monoclonal band is frequently found in the serum. Morphologically, SLVL cells have variable lymphoplasmacytic differentiation and show short membrane villi. Immunologically, the neoplastic lymphocytes are mature B cells, with moderate to strong expression of CD19, CD20, CD22, FMC-7, and HLA-Dr antigens and light-chain-restricted SmIg (usually IgM,k). CD11c and CD38 are positive in a variable proportion of cases, whereas CD5, CD23, CD25, and CD103 are usually negative. The bone marrow usually has a diffuse or nodular pattern of involvement. The spleen shows preferential infiltration of the white pulp, with involvement of the follicles, which may be surrounded or replaced by the lymphoma cells. Chromosome abnormalities are frequently found, with the preponderance of abnormalities involving chromosome 11q13, 7q, 17q, and 2p11. The disease course is usually stable or slowly progressive, and the main clinical problems are due to hypersplenism. The response to chemotherapy is in general poor, and splenectomy is the treatment of choice. Rarely, transformation to an aggressive large-cell lymphoma may occur.

Here we evaluate the expression of p26 on SLVL B cells by comparison with that observed on normal B lymphocytes and on neoplastic B cells from patients with other chronic B-cell neoplasms.

MATERIALS AND METHODS

Materials

We studied the expression of p26 on CD19(+) in lymphocytes from peripheral blood (PB), bone-marrow aspirates (BM), lymph nodes (LN), and spleen (SP) ob-

*Correspondence to: Margarida Lima, Service of Clinical Hematology, S. Antonio's Hospital, Largo Professor Abel Salazar 1, 4000 Porto, Portugal.

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TABLE I. Distribution of Patients and Samples According to the Diagnosis and Intensity of Expression of p26 on Normal and on Neoplastic B Cells*

Samples	Control individuals without evidence of B-cell neoplasms	Chronic B-cell lymphoproliferative disorders		
		SLVL (n = 24)	B-CLL (n = 59)	Other (n = 32)
Peripheral blood (n = 164)	4.0 ± 0.6 (3.0–4.9)[a] (n = 65)	4.8 ± 1.6 (n = 24)	8.3 ± 2.4 (n = 53)	7.8 ± 3.4 (n = 22)
Bone marrow (n = 66)	3.8 ± 0.9 (2.8–5.3) (n = 33)	6.2 ± 3.0 (n = 4)	8.4 ± 2.7 (n = 17)	10.6 ± 5.6 (n = 12)
Lymph nodes (n = 38)	3.4 ± 1.2 (2.0–5.7) (n = 25)	6.3 (n = 1)	7.5 ± 1.8 (n = 2)	15.4 ± 12.5 (n = 10)
Spleen (n = 3)	3.6 (n = 1)	3.4 ± 0.7 (n = 2)	nd	nd
Total (n = 271)	3.8 ± 0.9 (2.1–5.1) (n = 124)	4.9 ± 1.9 (n = 31)	8.3 ± 2.5 (n = 72)	10.6 ± 7.9 (n = 44)

*Results are expressed as log units of mean fluorescence intensity. n, number of individuals or samples in each group.
[a]Mean ± 1 standard deviation (95% confidence limits).

tained from control adult individuals and patients with chronic B-cell neoplasms (Table I). Control PB were from regular blood donors. Control BM aspirates were obtained from patients studied for cytopenias, nonhematological neoplasias, and Hodgkin's disease, whose BM was considered normal by cytological, histological, and immunological criteria. Control LN and SP were from patients with nonhematological neoplasias and infectious diseases. One hundred and forty-seven samples from 115 patients with B-cell neoplasms were analyzed: 24 patients with SLVL, 57 patients with B-cell chronic lymphocytic leukemia (B-CLL) as well as 2 patients with B-cell prolymphocytic leukemia (included in the same group for analysis), and 32 patients with other chronic B-cell lymphoproliferative disorders (see also Table I). All lymphoproliferative disorders were properly characterized by cytological, cytochemical, immunophenotyping, and histological studies, and none of them had been previously treated.

Sample Collection and Processing

Peripheral blood and BM samples were collected into EDTA-containing tubes. Lymph-node cells and splenocytes were obtained by gently teasing a representative piece of the organ in phosphate-buffered saline (PBS) containing 0.2% of bovine serum albumin (BSA, Sigma, St. Louis, MO) and 9 mM EDTA (PBS-BSA-EDTA, pH 7.2), using a pair of surgical pincers. Debris was removed by sedimentation and cells were washed once in PBS-BSA-EDTA and adjusted to the appropriate cell concentration before staining.

Flow Cytometric Quantification of p26

Quantification of p26 was performed by flow cytometry, using the Fix and Perm® reagent kit (An Der Grub Bioresearch GmbH, Austria) for cell fixation and permeation. Briefly, cells were incubated in the presence of mouse anti-human CD19 conjugated with phycoerithrin (Dakopatts, UK), washed twice, and fixed with Fix and Perm reagent A (An Der Grub Bioresearch GmbH, Austria). Fixed cells were pelleted by centrifugation, washed once, and incubated with mouse anti-human BCL-2 protein conjugated with fluorescein (Dakopatts) in the presence of Fix and Perm reagent B. After washing, cells were resuspended in PBS-BSA-EDTA and analyzed in an MCL-XL flow cytometer (Coulter Electronic, Inc., Hialeah, FL). At least 2,500 events were acquired after gating the CD19(+) lymphocyte population. The intensity of expression of p26 was measured with detectors and amplifiers set on a logarithmic scale. The results are expressed as log units of mean fluorescence intensity.

Statistical Analysis

Data were analyzed by using Student's t-test.

RESULTS

The mean fluorescence intensity of p26 on SLVL B cells (4.9 ± 1.9) (mean ± 1 standard deviation) was slightly higher than that observed in normal mature B lymphocytes (3.8 ± 0.9) ($P < 0.005$). However, levels of p26 on SLVL B cells were significantly lower than those observed in B-CLL (8.3 ± 2.5) ($P < 0.005$) and in other chronic B-cell malignancies (10.9 ± 7.6) ($P < 0.005$) (Table I and Fig. 1). Twelve out of 24 patients with SLVL (50%) showed normal levels of p26, whereas this occurred in only 6 out of 59 patients with B-CLL (10%) and 7 out of 32 patients with other chronic B-cell lymphoproliferative disorders (21%).

One of the patients included in this series was subsequently splenectomized with success. Disease progression occurred after a 2-year period of stability, and at that time there were cytological changes in peripheral blood suggesting transformation to a large-cell lymphoma. At the time of disease progression, peripheral blood B cells expressed very high levels of p26 (log mean fluorescence intensity 26.9), at values that are never observed in SLVL and that only rarely occur in other B-cell neoplasms. The patient was refractory to chemotherapy and ultimately died of the disease.

DISCUSSION

BCL-2 was first described as the oncogene involved in the genesis of follicular center-cell lymphomas [2]. Sub-

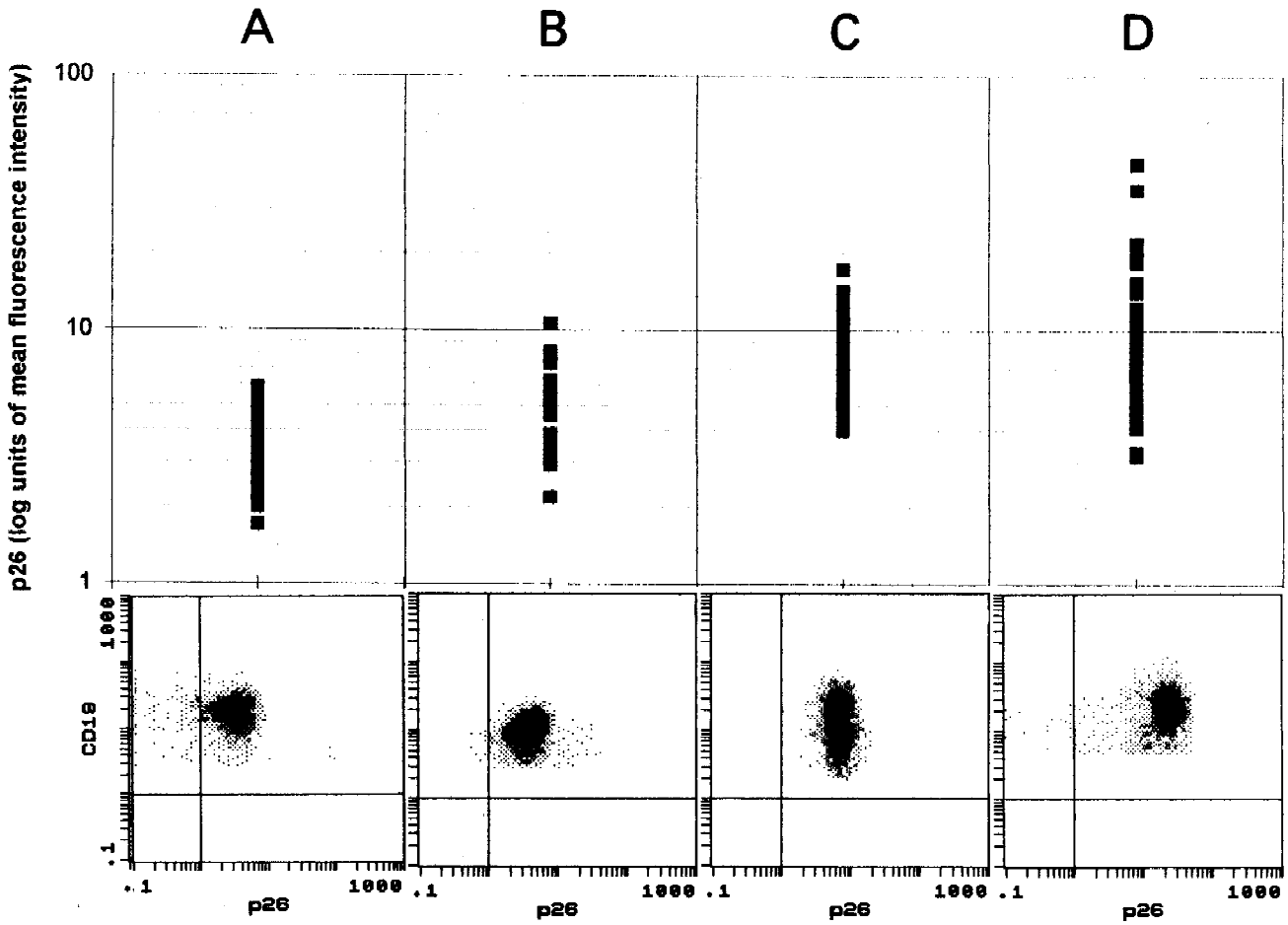

Fig. 1. Expression of p26 on mature CD19(+) lymphocytes from control individuals (A), splenic lymphoma with villous lymphocytes (B), chronic B-cell lymphocytic leukemia (C), and other peripheral B-cell neoplasms (D). Illustrative examples of CD19/p26 dot plots are shown in each case.

sequent studies showed that many B-cell neoplasms that lack the t(8;14) translocation also have high levels of BCL-2 protein [3,4]. By now, there is enough evidence to hypothesize that BCL-2-induced extended cell survival plays a primary role in a multioncogenesis model of lymphomagenesis [1]. SLVL is a chronic B-cell malignancy that was recently characterized based on clinical and laboratorial grounds [6–8]. Although it was described for the first time only a few years ago, the increasing accuracy in diagnosis is showing that it represents a frequent entity in the spectrum of chronic B-cell malignancies. The pathogenesis of SLVL remains to be elucidated. SLVL B cells probably originate from splenic marginal-zone cells, and the relationship between SLVL and splenic marginal-zone cell lymphoma has been investigated. Previous studies revealed that some cases show the t(11;14) translocation and rearrangement of the BCL-1 locus at levels that appear diverse from those of mantle-cell lymphoma, with concomitant expression of cyclin D1 transcript [7,9]. However, reports on the role of other oncogenes are scarce, and to the best of our knowledge the expression of p26 in SLVL has not been previously characterized. Our results demonstrate for the first time that SLVL shows relatively low levels of p26 when compared with other chronic B-cell lymphoproliferative disorders, suggesting that upregulation of BCL-2 expression is not a major event in the genesis of this B-cell neoplasm. Although the level of expression of p26 cannot be used on an individual basis to establish the differential diagnosis of other entities, this study corroborates the idea that SLVL represents a distinct disease. Lower levels of BCL-2 may account for the indolent disease course, without progression of lymphocytosis observed in the majority of cases. Upregulation of BCL-2 may be observed during disease transformation to a large-cell lymphoma.

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